

# Calf venous volume during stand-test after a 90-day bed-rest study with or without exercise countermeasure

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The objectives to determine both the contribution to orthostatic intolerance (OI) of calf venous volume during a stand-test, and the effects of a combined eccentric–concentric resistance exercise countermeasure on both vein response to orthostatic test and OI, after 90-day head-down tilt bed-rest (HDT). The subjects consisted of a control group (Co-gr,  $n=9$ ) and an exercise countermeasure group (CM-gr,  $n=9$ ). Calf volume and vein cross-sectional area (CSA) were assessed by plethysmography and echography during pre- and post-HDT stand-tests. From supine to standing (post-HDT), the tibial and gastrocnemius vein CSA increased significantly in intolerant subjects (tibial vein, +122% from pre-HDT; gastrocnemius veins, +145%;  $P < 0.05$ ) whereas it did not in tolerant subjects. Intolerant subjects tended to have a higher increase in calf filling volume than tolerant subjects, in both sitting and standing positions. The countermeasure did not reduce OI. Absolute calf volume decreased similarly in both groups. Tibial and gastrocnemius vein CSA at rest did not change during HDT in either group. During the post-HDT stand-test, the calf filling volume increased more in the CM-gr than in the Co-gr both in the sitting ( $+1.3 \pm 5.1\%$ , vs.  $-7.3 \pm 4.3\%$ ;  $P < 0.05$ ) and the standing positions ( $+56.1 \pm 23.7\%$  vs.  $+1.6 \pm 9.6\%$ ;  $P < 0.05$ ). The volume ejected by the muscle venous pump increased only in the CM-gr ( $+38.3 \pm 21.8\%$ ). This study showed that intolerant subjects had a higher increase in vein CSA in the standing position and a tendency to present a higher calf filling volume in the sitting and standing positions. It also showed that a combined eccentric–concentric resistance exercise countermeasure had no effects on either post-HDT OI or on the venous parameters related to it.

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Human exposure to zero gravity or simulated microgravity (head-down tilt bed-rest, HDT) leads to cardiovascular deconditioning which is mainly characterized by reduced exercise capacity and orthostatic intolerance (OI). The major haemodynamic changes related to OI after HDT are a reduction in baroreflex sensitivity (Convertino, 1991; Custaud *et al.* 2002), a lack of lower limb arterial vasoconstriction (Arbeille *et al.* 1995, 1998; Herault *et al.* 2000), increased calf venous distensibility (Convertino *et al.* 1988, 1989b; Louisy *et al.* 1997; Arbeille *et al.* 1998) and reduced plasma volume. The latter does not appear to be a major cause of OI, as it is found in many tolerant subjects (Greenleaf *et al.* 1989; Pavy-Le Traon *et al.* 1999; Millet *et al.* 2000).

Reduced baroreflex sensitivity may occur under several circumstances in the absence of OI. A lack of arterial vasoconstriction has not been formally identified as a main factor for OI episodes after HDT. Nevertheless, there is enough blood volume stored in the venous side in the standing position to reduce cardiac filling.

On this basis, we hypothesize that it is the increase in calf venous volume that plays a major role in determining OI. As a logical follow-on from this, blood pooling in the calf has been proposed as a factor that precipitates the development of syncope (Hargreaves & Muir, 1992). Nevertheless, in some patients the increase in calf volume after moving to the standing position did not correlate with the onset of a vasovagal syncope (Bellard *et al.* 2003).

This may be due to the fact that direct measurement of vein compliance in humans is still difficult. It can in fact only be estimated indirectly by plethysmography on the basis of changes in calf volume (Convertino *et al.* 1988, 1989b; Louisy *et al.* 1997, 2001). This method takes into account the main and secondary changes in both calf vein cross-sectional area (CSA) and tissue volume, depending on tissue liquid filtration. However, it does not make possible proper identification of the various vein and tissue components involved in the development of OI. The first aim of this study was to test the hypothesis mentioned above by more accurately evaluating the role of increased venous volume after prolonged bed-rest as a determining factor for OI. To do so, we got round the difficulties encountered in previous studies by combining the use of air plethysmography for measuring absolute and relative changes in calf volume with that of transverse echographic views of the calf for measuring deep and muscular vein CSA.

The European Space Agency programme, which selected this study, gave us the opportunity to perform the same measurements on a group of subjects who had undergone an explosive exercise countermeasure, combining concentric and eccentric muscle contractions. It is well known that specific cardiovascular countermeasures, such as exposure to lower body negative pressure, restore plasma volume, baroreflex sensitivity and lower limb vasoconstriction even after several weeks in HDT (Arbeille *et al.* 1992, 1995; Traon *et al.* 1995). On the contrary, explosive exercise countermeasures, which efficiently counteract muscle atrophy (Convertino *et al.* 1989a; Louisy *et al.* 1995), can restore plasma volume but not lower limb arterial vasoconstriction (Arbeille *et al.* 1992; Gharib *et al.* 1992). We postulate that explosive exercise training during bed-rest may prevent a large increase in venous pooling, and thus improve venous return. As increased leg venous volume exaggerates the effects of both hypovolaemia (Watenpaugh & Hargens, 1996) and baroreflex impairment (Engelke *et al.* 1996), a study of the effects of explosive exercise countermeasures on venous properties after bed-rest may be of help in understanding the relative contribution of the latter to the occurrence of OI after bed-rest. Determining whether or not combined eccentric–concentric resistance exercise countermeasures could affect the calf vein response to a stand-test after 90-day HDT was thus the second aim of this study.

## Methods

### Subjects

Eighteen healthy young male subjects participated in the 90-day –6 deg HDT at the MEDES medical facility (Toulouse, France). Before the bed-rest, the subjects were aged  $33.1 \pm 0.9$  years, with average heights and weights

of  $1.75 \pm 0.01$  m and  $71.1 \pm 1.1$  kg, respectively. All subjects passed the orthostatic tolerance test (10 min +80 deg head-up tilt test) performed during the selection process. They received a complete description of the experimental procedure before giving their written informed consent to the protocol approved by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Midi-Pyrénées (France). The entire protocol was in accordance with the declaration of Helsinki. None of the subjects was taking cardiovascular medication at the time of the study and all subjects were non-smokers. The subjects were randomly divided into two groups: nine control subjects (Co-gr), and nine countermeasure subjects (CM-gr) who performed a combined eccentric–concentric resistance exercise every 3 days. At a later stage, having evaluated the occurrence of OI after bed-rest, a further subdivision was made between tolerant and intolerant subjects ( $n = 9$  in both groups). The distribution between the Co-gr and CM-gr turned out to be equivalent.

### HDT programme

The study design consisted of a 15-day ambulatory control period followed by 90 days of bed-rest in the –6 deg head-down tilt position, followed by 15 days of post bed-rest recovery. During the bed-rest, the subjects remained in HDT continuously for all activities. The subjects were given a diet of  $2000 \pm 300$  kcal day<sup>–1</sup> with a sodium input of 3 g day<sup>–1</sup>. Water intake was limited to 3 l day<sup>–1</sup>. Energy (~600 kcal) and water (~1 l) supplements were offered to the CM-gr on training day. The subjects were supervised and monitored 24 h day<sup>–1</sup>. Room lighting was on between 07.00 and 23.00 h daily. All studies were conducted in a quiet room at a temperature of ~24°C.

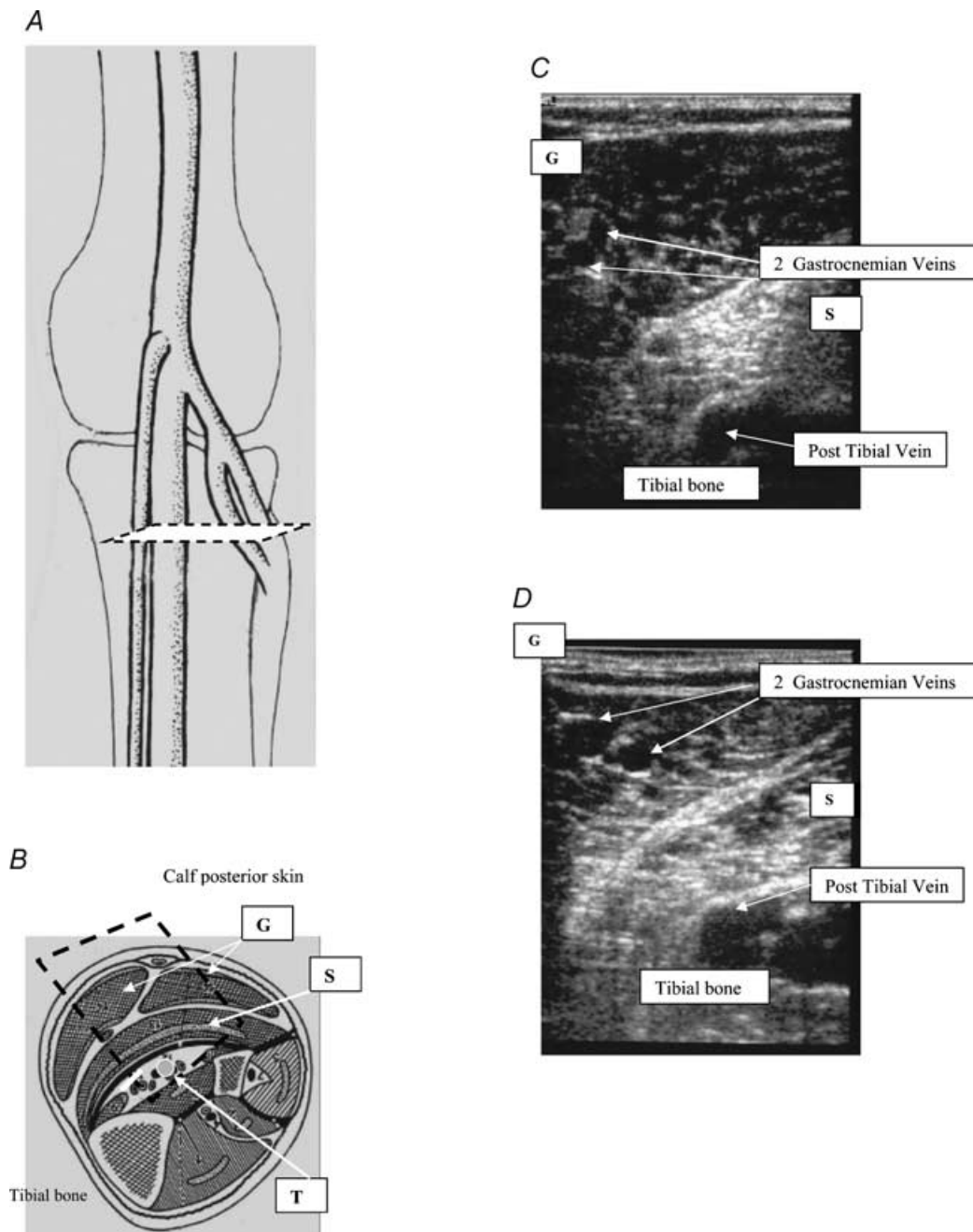
### Training protocol

The CM-gr took part in a resistance-exercise training programme, which consisted of series of intermittent, rhythmic, explosive efforts alternating between eccentric and concentric contractions on the flywheel exercise device. Two types of exercises can be performed on this device: the squat and the calf-press (Trappe *et al.* 2004). The former exercises the knee and hip extensor muscle groups, the latter exercises the ankle plantar flexors. Mean power during the concentric phase of the squat and calf-press activities was 555 W and 380 W, respectively. Training was composed of 29 sessions and was performed every 3 days, starting on day 5 of bed-rest. Progressive warm-ups preceded four sets of seven maximal concentric and eccentric repetitions in the squat, followed by four sets of 14 repetitions in the calf-press. Two minutes of rest were allowed between sets and 5 min between exercises. The subjects pushed with maximal concentric force until

almost full extension, paused for a moment just after the turning point and then attempted to stop the counteraction of the device (eccentric force). The total time of maximal muscle actions averaged 35 min.

### Plasma volume

Changes in plasma volume ( $\Delta PV$ ) were determined using the Dill & Costill (1974) equation, validated for bed-rest



**Figure 1. Anatomical arrangement of the veins examined, with an example of an echographic image**  
*A*, longitudinal posterior view of the upper part of the calf. Horizontal plane showing the level of the transverse echographic views. *B*, anatomical representation of the echographic transverse view. *C*, echographic transverse view in the supine position. Diameter of the posterior tibial vein, 0.5 mm; diameter of the gastrocnemian veins, 0.2 mm. *D*, echographic transverse view in the standing position. Diameter of the posterior tibial vein, 0.8 mm; diameter of the gastrocnemian veins, 0.4 mm. T, posterior tibial artery and vein; S, soleus muscle; G, gastrocnemius muscles.

by Johansen *et al.* (1997):

$$\Delta PV = 100[Hb_B(1 - Hct_A \times 10^{-2})] / [Hb_A(1 - Hct_B \times 10^{-2})] - 100,$$

where Hb are haemoglobin values and Hct are hematocrit values, measured before (suffix 'B') and during or after (suffix 'A') HDT. Hb and Hct were measured on venous blood samples, taken from a catheter inserted without compression into the antecubital vein of the left arm.

These measurements were taken once in the ambulatory control phase (day 1 before HDT), three times during HDT (days 3, 45 and 90), and once during recovery after HDT (day 9).

### Orthostatic tolerance test

Orthostatic tolerance was assessed using a tilt test on the day when the subjects stood up for the first time after HDT (day R0). The subject remained at rest in a supine position for 10 min; he was then tilted at 80 deg for 10 min on a tilt table equipped with a footplate. Heart rate and arm blood pressure were monitored beat-by-beat with a Portapres system (TNO, Biomedical Instrumentation Research Unit, Amsterdam, the Netherlands) and with an oscillometric device (Dynamap, Criticon, Tampa, FL, USA). The tilt tests were interrupted prematurely on the subject's request in case of discomfort, or if either (i) systolic blood pressure had decreased by  $\sim 30$  mmHg below the initial value or heart rate had increased by  $15 \text{ min}^{-1}$  in 1 min, or (ii) there

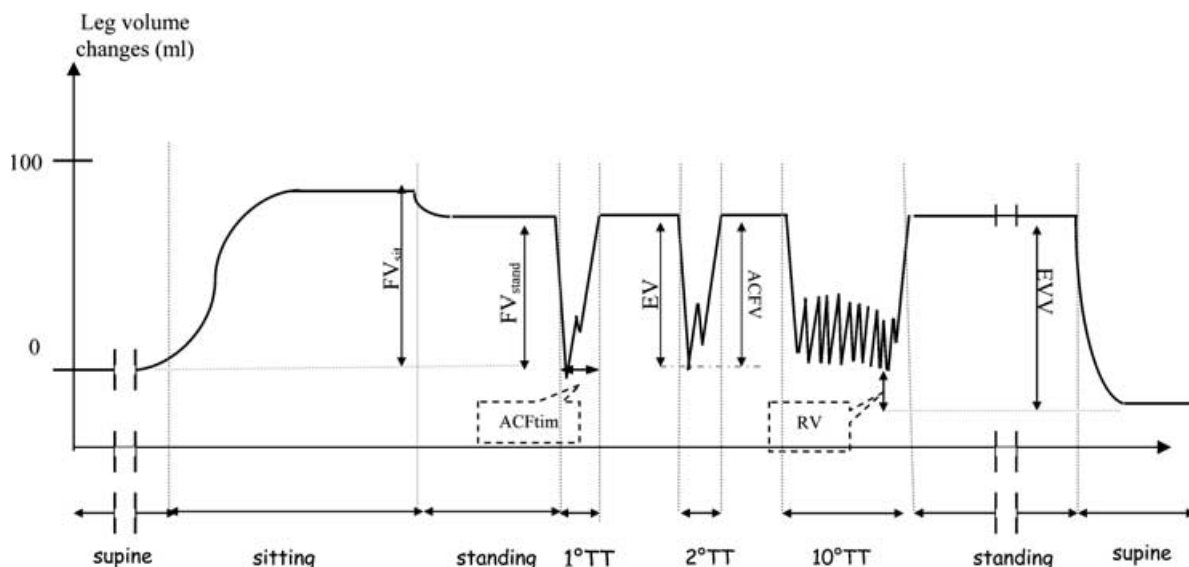
were signs of presyncope such as nausea, pallor, sweating, dizziness or visual disturbances.

### Plethysmography

Postural plethysmographic measurements were made on the right calf, using an Air Plethysmograph (APG-1000C & CP, ACI Medical, Sun Valley, CA, USA) and the technique previously described (Louisy *et al.* 2001). The volume of the right calf ( $V_0$ ) was estimated using the formula described by Thornton *et al.* (1992), after measuring calf circumference ( $c$ ) with a non-elastic measuring tape, carried out by the same individual at 3 cm intervals along the length of the calf.

### Echography

Calf vein CSA was measured using a 7.5 MHz ultrasound 'T-shaped probe' attached to the upper posterior level of the left calf by an adhesive patch, and connected to the echograph by a 2-m long cable (Esaote Challenge 2000, Florence, Italy). The ultrasound probe was placed in such a way as to visualize in a transverse cross-section the upper part of the tibial vein and one or two gastrocnemius veins depending on the subjects' anatomy. Echographic views were digitized and recorded continuously during the stand-test and processed using software designed by our laboratory (Fig. 1). The contours of the tibial and gastrocnemius veins were outlined on the images and the vein CSA expressed in  $\text{cm}^2$ .



**Figure 2.** Leg plethysmographic curve representing leg volume changes as a function of time

This figure explains the protocol used and the changes observed. The time at which each parameter was measured is indicated.  $FV_{sit}$ , filling volume in the sitting position (ml);  $FV_{stand}$ , filling volume in the standing position (ml); EV, ejected volume; ACFV, after contraction filling volume; ACF time, after contraction filling time; RV, residual volume; EVV, emptying venous volume; TT, tiptoe movements.

**Table 1. Tolerance time during the orthostatic tolerance test**

subjects	Co-group										CM-group									
	E1	F1	G1	I1	J1	C2	D2	E2	F2		A1	B1	C1	D1	G2	H2	I2	J2	K2	
BDC-15	10	10	10	10	10	10	10	10	10		10	10	10	10	10	8*	10	10	9*	
R0	8	5	8	10	10	10	10	3	10		10	10	10	5	7	10	5	7	8	

Individual data are reported. Time is in minutes. BDC-15 designates the control test carried out 15 days before the bed-rest; R0 designates the day when the subjects stood up for the first time after bed-rest. \*Subject intolerant at BDC-15 but tolerant at the selection tilt test.

### Stand-test protocol

Stand-tests were performed 3 days before bed-rest, and 2 and 6 days after bed-rest. All measurements were made in the morning, between 9.00 and 11.00 h. The measurement protocol used during the stand-test is illustrated in Fig. 2. After 30 min in the supine position, the subject was asked to sit up with his legs dangling for 5 min. At the end of 5 min, the subject was asked to stand up for 10 min.

Plethysmographic data were recorded continuously from the supine position to the end of the standing up position. The volume of the right calf was determined during the recumbent period of the stand-test protocol. The filling volume was determined during the 5 min in the sitting position ( $FV_{\text{sit}}$ ). It was measured again in the standing position, once the plateau had been attained ( $FV_{\text{stand}}$ ). The subject was then asked to perform two isolated tiptoe movements. The recorded decrease in volume at that time corresponds to the volume ejected from the venous reservoir as a consequence of calf contraction (EV). At the end of this contraction, a new plateau was reached, called the 'after contraction filling volume' (ACFV). The time needed to reach this plateau is the 'after contraction filling time' (ACF time). After this new plateau had been reached, the subject was asked to perform 10 tiptoe movements at the rate of 1 Hz. A decrease to a new steady state, called the residual volume (RV), was then observed. Then the subject remained in the standing position for 10 min. At the end of the stand-test the subjects returned to the supine position in order to determine emptying venous volume.

Volume changes were expressed as absolute values (ml) irrespective of initial leg volume. Because of inter-subject differences in leg volume, and variations in a given subject depending on different experimental situations, leg volume changes were expressed as a function of initial leg volume (i.e. in relative units,  $FV_{\text{sit or stand}}/V_0$ , in ml (100 ml tissue)<sup>-1</sup>).

Other parameters were calculated from the postural plethysmographic curve. The ejection fraction (EF) and the residual venous fraction (RVF) were calculated as follows:

$$EF = [(EV/FV_{\text{sit}}) \times 100] \quad (1)$$

$$RVF = [(RV/FV_{\text{sit}}) \times 100] \quad (2)$$

EF is an index of the ejection function of the calf muscle pump, which plays a key role in venous return. RVF is an index of venous emptying function.

### Statistical analysis

Values were expressed as mean  $\pm$  s.e.m., and changes were considered statistically significant for  $P < 0.05$ . Data were analysed using non-parametric tests because of the number of subjects. Comparisons between the Co-gr and CM-gr, and between tolerant and intolerant subjects, were made using the Mann Whitney *U* test for unpaired variables. Two periods within the same group were compared using the Wilcoxon test for paired data.

## Results

### Orthostatic tolerance

Table 1 shows the time during which the subjects remained upright during the tilt test (orthostatic reference test) at day 15 of HDT (HDT-15) and R0. Four of the nine subjects in the Co-gr and five of the nine subjects in the CM-gr did not finish the tilt test and were considered to be intolerant.

### Body mass

The evolution over time of body mass is reported in Fig. 3. Body mass decreased during HDT in both groups. Its decrease became significant in both groups (Co-gr,  $-0.91 \pm 0.18$  kg; CM-gr,  $-0.62 \pm 0.28$  kg) after only 2 days in bed. In the Co-gr, it continued until the end of bed-rest (day R0,  $-2.75 \pm 1.06$  kg). The decrease in overall body mass during HDT in the CM-gr ( $-0.75 \pm 0.19$  kg at day R0) was less than in the Co-gr. In the CM-gr, a steady level was maintained from HDT-15 until HDT-58. In each group, the decrease in body mass was similar in intolerant and tolerant subjects.

### Plasma volume

The plasma volumes calculated, which are shown in Fig. 4, decreased significantly at HDT-3 and HDT-90 compared with the control values before bed-rest. No difference was

found between either the Co-gr and CM-gr or between intolerant and tolerant subjects.

### Calculated calf volume

The calf volume values and all related parameters are shown in Table 2 (A, Co-gr vs. CM-gr; B, tolerant subjects vs. intolerant subjects). Absolute calf volume at rest decreased both significantly and progressively in both groups during bed-rest from day HDT-3 until day HDT-81 (Co-gr,  $-17.4 \pm 1.3\%$ ; CM-gr,  $-14.7 \pm 0.5\%$ ), as shown in Fig. 5. No significant differences were found between either the Co-gr and CM-gr or between tolerant and intolerant subjects.

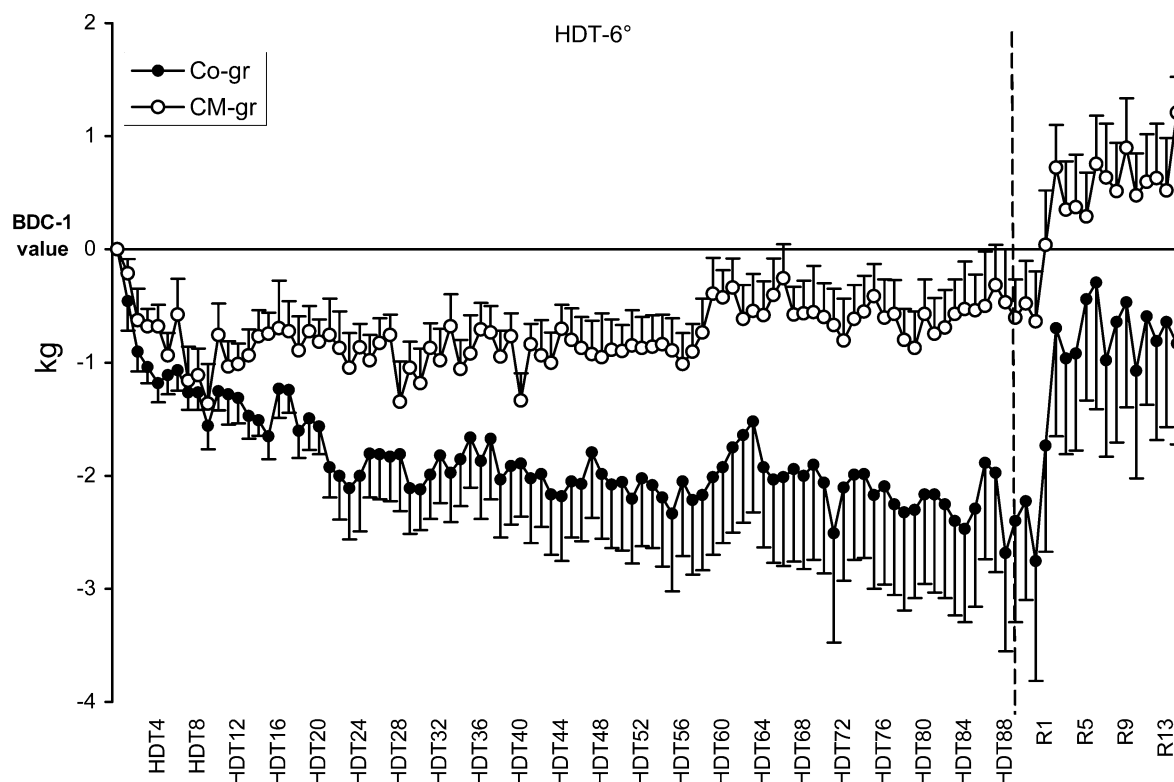
A comparison of plethysmographic data between the Co-gr and CM-gr is shown in Table 2A. No counter-measure effect was observed on  $FV_{sit}$  during the days 1 and 6 after bed-rest (R+1 and R+6, respectively) stand-tests.  $FV_{stand}$  was significantly higher in the CM-gr than in the Co-gr at the R+1 stand-test, and similar to the pre-HDT test in the Co-gr. During the stand-tests, EV was maintained and EF increased in the CM-gr. These variables decreased significantly in the Co-gr. RV and RVF were not altered by the 90 days of bed-rest in either group.

A comparison of plethysmographic data between intolerant and tolerant subjects is shown in Table 2B. The most important differences concerned the calf filling

volumes reported in Fig. 6.  $FV_{sit}$  did not vary significantly between groups ( $P = 0.20$ ), yet it tended to increase in intolerant subjects, and decrease in tolerant subjects.  $FV_{stand}$  increased during bed-rest in a similar manner in both groups ( $P = 0.69$ ). No differences were observed as far as the other parameters were concerned.

### Vein cross-sectional area

Tibial and gastrocnemius vein CSA in the supine position were similar before and after bed-rest (Table 3). The CSA of these veins increased significantly from the supine to standing positions, both before and after bed-rest. In the latter case, the CSA increase from supine to standing was greater in intolerant subjects than in tolerant subjects. Furthermore, the increase in the CSA of intolerant subjects was greater after bed-rest than before, as shown in Figs 7 and 8. Seven of the eight intolerant subjects effectively increased their vein CSA from the supine to the standing position by more than 10% (10–100%) at the post-HDT stand-test compared with pre-HDT. On the other hand, eight of the 10 tolerant subjects maintained or reduced their vein CSA (from the supine to the standing position) from the pre- to post-HDT stand-tests. However, as the proportion of intolerant subjects was the same in both the Co-gr and CM-gr (see Table 1), no differences in CSA increase were observed between the two groups.



**Figure 3. Changes in body weight during and after bed-rest, progression over time**

On the x-axis, HDT is head-down tilt during the bed-rest period and R is recovery. BDC-1, control value before bed-rest; Co-gr, control group; CM-gr, countermeasure group.

**Table 2. Pre- and post-bed-rest values for the plethysmographic data**

A	BDC-2		R+1		R+6	
	Co-gr	CM-gr	Co-gr	CM-gr	Co-gr	CM-gr
FV <sub>sit</sub> (ml (100 ml) <sup>-1</sup> )	3.86 ± 0.3	4.41 ± 20	3.53 ± 0.2	*4.42 ± 0.20	3.83 ± 18	*4.93 ± 0.29
FV <sub>stand</sub> (ml (100 ml) <sup>-1</sup> )	3.64 ± 0.5	3.57 ± 0.57	3.67 ± 0.3	*4.79 ± 0.37	3.94 ± 0.44	4.49 ± 0.54
EV (ml (100 ml) <sup>-1</sup> )	1.82 ± 0.3	1.70 ± 0.41	1.47 ± 0.2	1.83 ± 0.20	1.79 ± 0.30	1.57 ± 0.22
FE (%)	45.57 ± 5.9	37.69 ± 7.92	#42.67 ± 5.7	#43.02 ± 4.50	41.60 ± 5.59	30.73 ± 4.45
RV (ml (100 ml) <sup>-1</sup> )	1.37 ± 0.2	1.56 ± 0.33	1.63 ± 0.4	1.87 ± 0.39	1.17 ± 0.29	2.00 ± 0.48
RVF (%)	0.36 ± 0.1	0.37 ± 0.09	0.46 ± 0.1	0.43 ± 0.09	0.30 ± 0.07	0.39 ± 0.09
ACFV (ml (100 ml) <sup>-1</sup> )	1.88 ± 0.3	1.80 ± 0.40	1.48 ± 0.2	1.89 ± 0.18	1.67 ± 0.21	1.58 ± 0.20
ACF time (s)	7.48 ± 0.8	6.93 ± 0.47	8.81 ± 1.2	#8.86 ± 0.66	#11.23 ± 1.11	* #8.63 ± 0.65
EVV (ml (100 ml) <sup>-1</sup> )	3.88 ± 0.5	4.29 ± 0.33	4.45 ± 0.3	#5.45 ± 0.47	2.46 ± 0.09	4.94 ± 0.53

B	BDC-2		R+1		R+6	
	Tolerant	Intolerant	Tolerant	Intolerant	Tolerant	Intolerant
V <sub>0</sub> (ml)	2416 ± 93.9	2401 ± 52.7	2015 ± 64.4	2049 ± 47.1	2173 ± 71.9	2188 ± 56.2
ΔPV (%)	0 ± 0	0 ± 0	#-6.69 ± 2.11	#-5.99 ± 2.25	#12.62 ± 2.96	#11.19 ± 3.36
FV <sub>sit</sub> (ml (100 ml) <sup>-1</sup> )	4.176 ± 0.30	4.087 ± 0.19	3.722 ± 0.30	4.233 ± 0.17	3.974 ± 0.20	4.784 ± 0.34
FV <sub>stand</sub> (ml (100 ml) <sup>-1</sup> )	3.46 ± 0.54	3.753 ± 0.50	4.154 ± 0.45	4.306 ± 0.35	3.952 ± 0.39	4.474 ± 0.58
EV (ml (100 ml) <sup>-1</sup> )	1.907 ± 0.39	1.612 ± 0.29	1.724 ± 0.24	1.579 ± 0.14	1.692 ± 0.29	1.67 ± 0.23
FE (%)	43.58 ± 7.48	39.68 ± 6.66	46.75 ± 6.17	38.94 ± 3.23	38.44 ± 5.58	33.89 ± 5.10
RV (ml (100 ml) <sup>-1</sup> )	1.21 ± 0.17	1.715 ± 0.31	1.627 ± 0.32	1.865 ± 0.42	1.347 ± 0.41	1.814 ± 0.43
RVF (%)	0.284 ± 0.03	0.445 ± 0.09	0.439 ± 0.09	0.446 ± 0.10	0.322 ± 0.09	0.361 ± 0.07
ACFV (ml (100 ml) <sup>-1</sup> )	1.969 ± 0.37	1.707 ± 0.33	1.698 ± 0.24	1.665 ± 0.14	1.635 ± 0.22	1.615 ± 0.20
ACF time (s)	6.894 ± 0.63	7.517 ± 0.64	#9.056 ± 1.05	8.611 ± 0.87	#9.856 ± 1.12	#10 ± 0.91
EVV (ml (100 ml) <sup>-1</sup> )	3.719 ± 0.44	4.454 ± 0.41	#4.787 ± 0.38	5.112 ± 0.45	2.416 ± 0.09	4.752 ± 0.53

A, refers to the control (Co-gr) and countermeasure (CM-gr) groups. B, refers to the tolerant and intolerant subjects. Data are given as mean ± S.E.M. FV<sub>sit</sub>, filling volume in the sitting position; FV<sub>stand</sub>, filling volume in the standing position; EV, ejected volume; EF, ejected fraction; RV, residual volume; RVF, residual venous fraction; ACFV, after contraction filling volume; ACF time, after contraction filling time; EVV, emptying venous volume; BDC-2, control measurements on day 2 before bed-rest; R+1 and R+6, days 1 and 6, respectively, after bed-rest. \*Significant difference between groups,  $P < 0.05$ ; #significant difference within groups with respect to BDC-2-value.

## Discussion

The 90-day HDT study showed that in intolerant subjects the vein CSA increased more in the standing position. This was associated with a tendency to have a higher calf filling volume in the sitting and standing positions. This finding concords with the hypothesis that increased venous pooling at the calf level plays a major role in the determination of OI. Furthermore, it showed that a combined eccentric–concentric resistance exercise countermeasure on the flywheel exercise device had no effect on OI after the HDT, or on the venous parameters related to it.

### Calf vein CSA changes in relation to orthostatic intolerance

This study showed that the increase in changes in calf vein CSA, as measured by echography in a post-HDT stand-test as compared with pre-HDT, was strongly related to the occurrence of OI after the HDT (sensitivity, 0.88;

specificity, 0.80). The increase in vein CSA in the intolerant subjects was similar for both the tibial vein, which is a major vessel, and the gastrocnemius veins (muscular veins), changes in which may be influenced by the volume and tone of the surrounding muscle mass. This observation suggests that the changes in muscle mass, and probably hydration, did not affect venous pooling. This supports the notion that the changes induced by HDT in the properties of veins are not linked to either muscle mass or tone.

The mechanical properties of the vein wall may change during HDT. Unfortunately neither morphological analysis of the components of the vein wall nor evaluation of vein response to vasoactive drugs were performed in this study. It is thus difficult to verify this hypothesis. In addition, there are no experimental data for either humans or animals to support this idea.

Until now, regulation of calf deep vein distensibility by the sympathetic nervous system has not been demonstrated in either animal or human studies. Purdy

*et al.* (1998) reported no significant effect of rat hindlimb suspension on maximal vein response to noradrenaline (jugular and femoral). However, Sayet *et al.* (1995) revealed modifications in the properties of rat vena cava. Alterations to noradrenaline-induced contraction by decreased affinity to  $\alpha_{1B}$  adrenoreceptors was observed after hindlimb suspension. Moreover, after 21 days of rat hindlimb suspension, the small mesenteric veins had a decreased response to noradrenaline (Dunbar *et al.* 2000). As such results, in animals, are not available for the calf, it would be dangerous to extrapolate them as a way of explaining the modifications observed in the present study. In humans, it remains unclear to what degree the sympathetic system modulates calf vein compliance, as sympathetic innervation is mainly studied at the arterial level. Two studies have shown dissociation between sympathetic activation and arterial vasoconstriction. Dissociation between the increase in sympathetic activity and modifications in leg vascular resistance has been observed by Jacob *et al.* (2000). They reported no changes in the vascular tone of the legs in response to sympathetic activation, as measured by noradrenaline spillover. Similar results were obtained by microneurography (Imadojemu *et al.* 2001). Moreover, Halliwill *et al.* (1999) specifically studied calf veins and demonstrated no effects of sympathetic activation on calf venous compliance in humans.

A decrease in arterial vasoconstriction may also play a role in venous pooling. A significant lack of vasoconstriction and absence of leg flow reduction was found almost systematically in intolerant subjects as compared with tolerant subjects in previous studies (Arbeille *et al.* 1998; Herault *et al.* 2000). This lack of arterial vasoconstriction results in a pressure and volume increase in venules which in turn causes an increase in venous blood filling (Rowell, 1993).

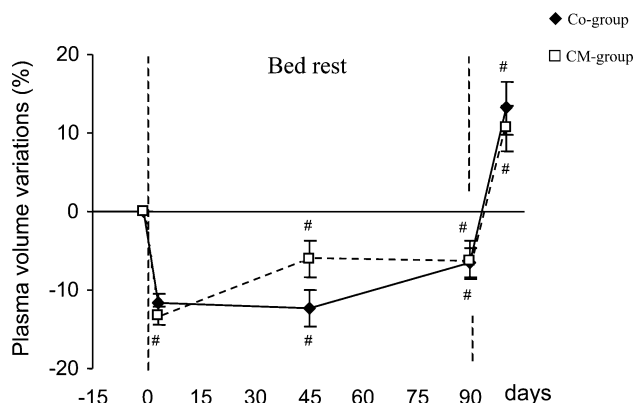
Some authors have reported that the distal leg arteries of rats suspended by their tails become more distensible, given that the arterial wall must have become thinner (Delp *et al.* 2000). This observation and the results of our study suggest that both arteries and veins at the calf level might become more distensible secondary to vascular remodelling induced by chronic changes in blood flow and pressure during HDT.

Intolerant subjects were equally distributed between the Co-gr and CM-gr. Thus, the differences observed in the changes in calf vein CSA after bed-rest did not have an effect on the comparison between the Co-gr and CM-gr. Although the CM-gr had less muscle atrophy than the Co-gr (Alkner & Tesch, 2002), the alterations in venous CSA in the stand-test were similar to those of the Co-gr. This demonstrates that the explosive exercise counter-measure used in this project had no effect on the change in vein CSA during the stand-test, and suggests that the changes in vein CSA during the stand-test were not closely linked to muscle mass or tone reduction.

#### Calf volume changes during the stand-test in relation to orthostatic intolerance

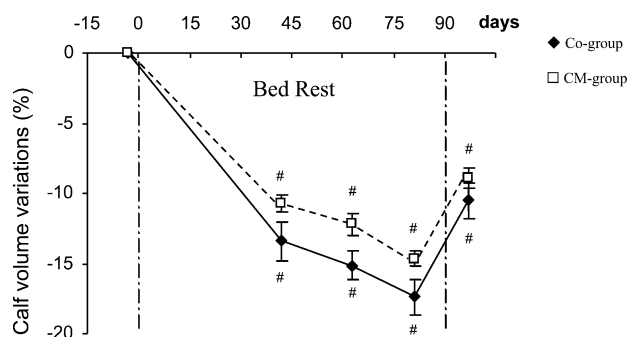
By measuring the changes in calf volume while sitting and standing, plethysmography indirectly assessed the filling capabilities of veins and the calf. As the plethysmographic measurements were taken continuously during the stand-test, we were able to eliminate the variations in calf volume caused by abrupt muscle mass displacement. Thus, the alterations observed are due only to blood stagnation in the venous compartment and to capillary filtration.

During standing, the trend towards higher calf filling volume in the intolerant subjects corroborates the greater changes in calf vein CSA observed in this group. Increased capillary permeability, caused by leg dehydration, extravascular pressure and loss of muscle mass, has been reported during previous bed-rest



**Figure 4. Plasma volume variations vs. BDC-1**

Plasma volume variations calculated in the Co-group ( $\blacklozenge$ ,  $n = 9$ ) and the CM-group ( $\square$ ,  $n = 9$ ). Values are mean  $\pm$  S.E.M. #  $P < 0.05$  vs. BDC-1-values in each group, respectively.



**Figure 5. Percentage variation in calf volume vs. BDC-2**

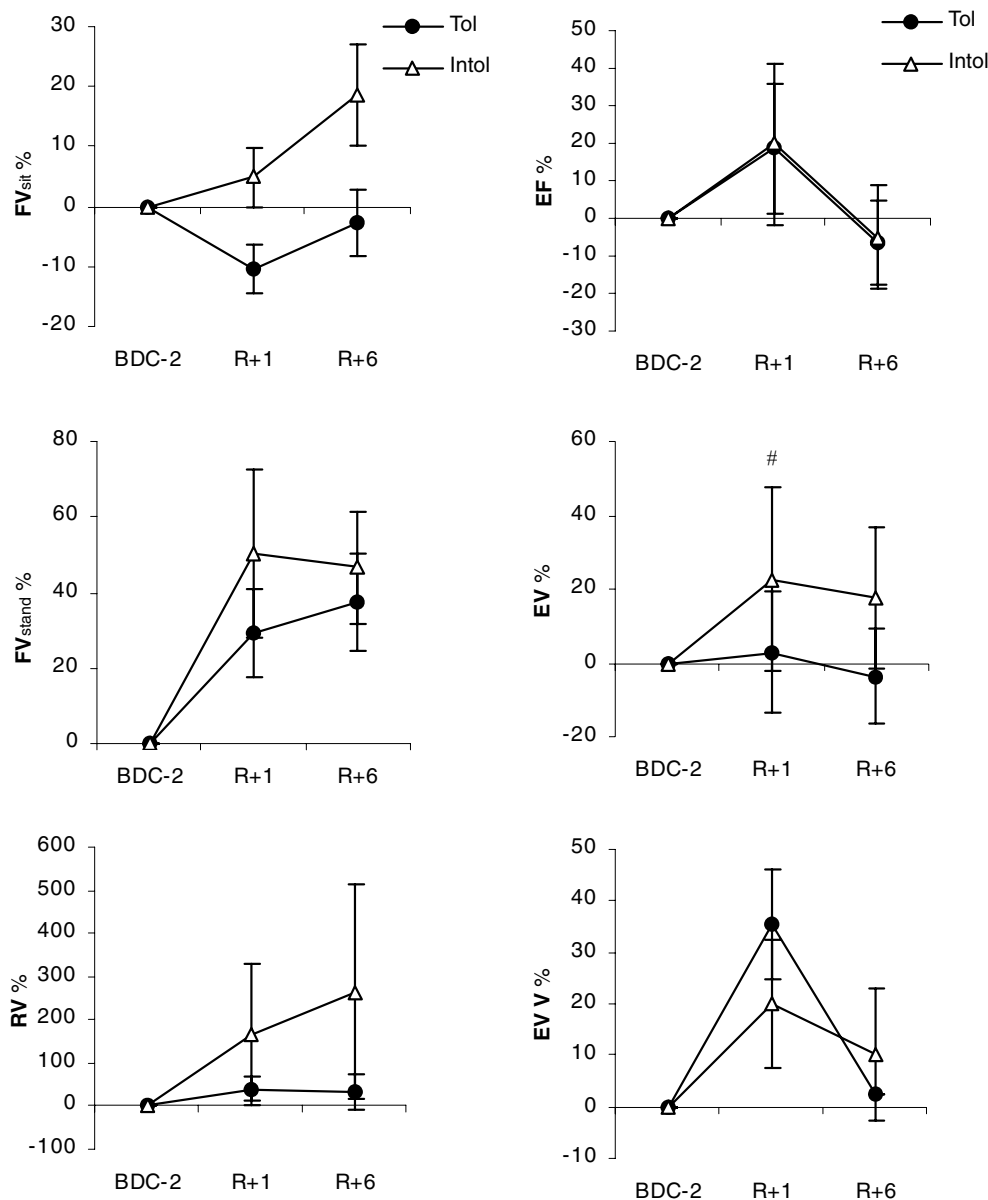
Percentage variation in calf volume calculated in the Co-group ( $\blacklozenge$ ,  $n = 9$ ) and in the CM group ( $\square$ ,  $n = 9$ ). Values are mean  $\pm$  S.E.M., #  $P < 0.05$  vs. BDC-1-values in each group, respectively.



periods or at the end of spaceflight (Christ *et al.* 2001). These modifications in capillary permeability may explain the greater calf volume filling observed in intolerant subjects. It is nevertheless difficult to evaluate the relative contribution of tissue filtration and secondary vein filling to the changes in calf volume, because the vein CSA is small compared with the calf CSA, as well as because of muscle contraction during the stand-test.

The continuous measurements taken using air plethysmography made it possible to evaluate the

efficiency of the calf venous muscle pump. As the intolerant subjects did not have a higher alteration in EF than that observed in the tolerant subjects, this variable might initially seem to be unrelated to OI. Although the efficiency of this muscle pump increased in the CM-gr, this group did not have better orthostatic tolerance than the Co-gr. However, at the same time, the CM-gr had a higher calf venous filling volume in the standing position. This higher blood volume stagnation in the legs may have induced the lack of improvement in orthostatic tolerance



**Figure 6. Percentage changes in plethysmographic measurements vs. BDC-2**

Percentage changes in plethysmographic measurements.  $FV_{sit}$ , filling volume in the sitting position;  $FV_{stand}$ , filling volume in the standing position; RV, residual volume; EF, ejected fraction; EV, ejected volume; EVV, emptying venous volume; Tol, tolerant subjects at the R0 tilt test; Intol, intolerant subjects at the R0 tilt test. Values are mean  $\pm$  S.E.M.,

\*Significant difference between groups,  $P < 0.05$ ; #significant difference vs. BDC-2-values,  $P < 0.05$ .

**Table 3. Mean tibial and gastrocnemius vein cross-sectional areas in the supine position and at the end of the stand-test period**

Cross-sectional areas (cm <sup>2</sup> )	BDC-2		R+1	
	Co-gr	CM-gr	Co-gr	CM-gr
Tibial supine	0.31 ± 0.05	0.26 ± 0.05	0.26 ± 0.03	0.23 ± 0.06
Tibial stand	0.68 ± 0.04	0.61 ± 0.07	0.65 ± 0.07	0.66 ± 0.10
Gast supine	0.08 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
Gast stand	0.17 ± 0.04	0.11 ± 0.03	0.15 ± 0.04	0.12 ± 0.03
	Tolerant	Intolerant	Tolerant	Intolerant
Tibial supine	0.30 ± 0.06	0.26 ± 0.03	0.30 ± 0.05	0.20 ± 0.04
Tibial stand	0.66 ± 0.04	0.63 ± 0.06	0.68 ± 0.08	0.63 ± 0.10
Gast supine	0.06 ± 0.01	0.07 ± 0.02	0.04 ± 0.01	0.05 ± 0.01
Gast stand	0.17 ± 0.03	0.11 ± 0.03	0.12 ± 0.03	0.15 ± 0.03

BDC-2, control measurements on day 2 before bed-rest; R+1, day 1 after bed-rest; Co-gr, control group; CM-gr, countermeasure group; Gast, gastrocnemius veins. Data are given as mean ± S.E.M. Cross-sectional area is expressed in cm<sup>2</sup>.

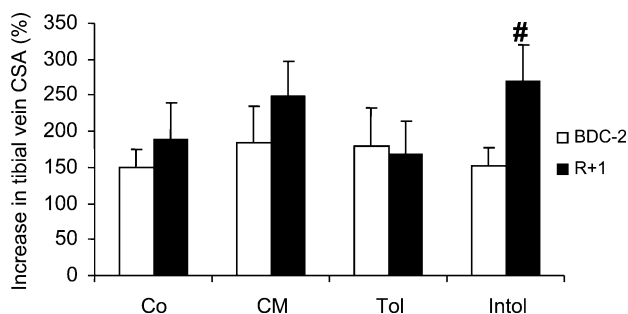
but may also provide more blood to be expelled by the muscle venous pump. Consequently, the ratio between the blood volume expelled and the amount of blood confined in the leg venous compartment is probably not high enough to induce an improvement in orthostatic tolerance.

### Effects of explosive exercise countermeasure on calf venous properties

As mentioned above, the CM-gr had a higher calf filling volume in the standing position. As athletes are characterized by a higher capillary filtration rate in their calves (Hildebrandt *et al.* 1993), this may suggest that more liquid in the distal venous network and higher capillary filtration may play a role in increasing the amount of liquid stored in the leg, and thus increase filling volume. Alternatively, an increase in calf filling volume may be the result of increased muscle capillarity. Capillary density

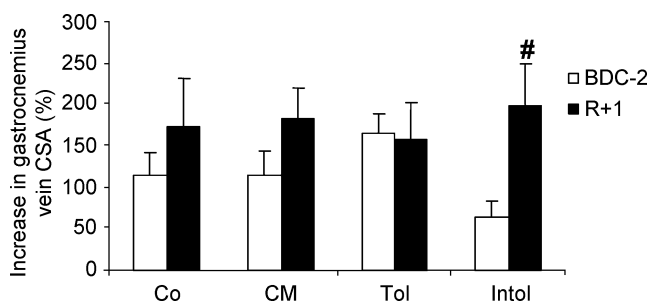
has been reported to be unchanged after 42-day bed-rest without countermeasure (Ferretti *et al.* 1997) and to be increased by a resistance exercise programme (Green *et al.* 1999; Hostler *et al.* 2001; McCall *et al.* 1996). Capillary density was not measured in this study; however, as the CM-gr took part in a resistance exercise training programme, it is logical to suppose that an increase in capillary density in the CM-gr might have contributed to the increase in calf filling volume.

Despite this improvement in the efficiency of the calf venous pump, the CM-gr did not have higher orthostatic tolerance than the Co-gr at the end of the 90-day HDT. This study corroborates several previous studies, which demonstrated that resistance training, regardless of the protocol applied, does not improve orthostatic tolerance (Tatro *et al.* 1992; McCarthy *et al.* 1997; Panton *et al.* 2001). Other studies, using intensive isotonic and isokinetic exercise as a countermeasure (Greenleaf *et al.* 1989), have demonstrated that a combination of these countermeasures, though not improving OI, could at



**Figure 7. Percentage increase in tibial vein CSA, standing position compared to supine position**

Calculated in the control group (Co,  $n = 9$ ), in the countermeasure group (CM,  $n = 9$ ), in the tolerant subjects (Tol;  $n = 9$ ) and intolerant subjects (Intol;  $n = 9$ ). Values are mean ± S.E.M., # $P < 0.05$ , significant difference vs. BDC-2-values.



**Figure 8. Percentage increase in gastrocnemius vein CSA, standing position compared to supine position**

Calculated in the control group (Co,  $n = 9$ ), in the countermeasure group (CM,  $n = 9$ ), in the tolerant subjects (Tol;  $n = 9$ ) and intolerant subjects (Intol;  $n = 9$ ). Values are mean ± S.E.M.,  $P < 0.05$ , #significant difference vs. BDC-2-values.

least restore plasma volume. In the present study, the loss of plasma volume, calculated from variations in Hb and Hct or indirectly evaluated from left ventricular internal volume, was similar to that observed during previous periods of bed-rest of different durations (Gharib *et al.* 1992; Arbeille *et al.* 1995, 1999, 2001; Traon *et al.* 1998; Millet *et al.* 2000). This observation supports the hypothesis that plasma volume may play a role in the onset of OI. As already described by Greenleaf *et al.* (1989), this study confirms the fact that resistance exercise is not sufficient to improve post-HDT OI and counterbalance the loss of plasma volume induced by microgravity. Endurance exercise, because of its effects on plasma volume, is more likely to reduce cardiovascular deconditioning induced by bed-rest (Fortney *et al.* 1996).

## Conclusions

In conclusion, the results of this study demonstrate that (i) the higher calf vein CSA observed during the stand-test after bed-rest is related to the occurrence of OI, but is independent of the exercise countermeasure; (ii) there is a trend towards an increase in blood pooling in the calf and improved calf muscle pump efficiency in relation to the exercise countermeasure; (iii) the explosive exercise countermeasure has no effects on either OI or the venous parameters related to it; and (iv) the combination of plethysmographic and echographic measurements of venous filling characteristics made possible a better understanding of the mechanisms that initiate OI episodes after exposure to microgravity.

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